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MOLECULAR STRATEGIES FOR CHARACTERIZATION OF FUNGAL ISOLATES FROM URUGUAYAN RICE FIELDSCAPDEVIELLE, F. ¹; FEDERICI, M.T. ¹; SOLARES, E.; BRANDA, A. ¹; AVILA, S. ¹ National Institute of Agricultural Research (INIA), Uruguay

Sustainability of the rice production, which has expanded both the area and the productivity per hectare in the last years, is one of the most important bases of the Uruguayan rice industry. However, with the increase of the rice frequency in the agricultural-pastures rotations, some emergent diseases have been identified as the main risks for crop intensification. Fungal samples corresponding to *Rhizoctonia* and *Sclerotium* species associated with the culm and sheath complex of diseases, often inaccurately identified using morphological characters, were collected during 2000 and 2001 crop seasons from rice plants and soil in different rice fields throughout the range of rice crops in Uruguay. Fungal DNA samples were analyzed either by PCR amplification using combinations of ITS primers and species-specific primers (GMROS-2 and GMROS-6 for *R. oryzae-sativae*, GMRS-3 and GMRS-4 for *R. solani*, and GMRO-3 for *R. oryzae*), based on Johanson et al (1998). The combination GMRS-4/ ITS1 was also included to compare amplified sequences from *Sclerotium* isolates. On the other hand, a basic step in breeding for blast durable resistance is the identification of population structure for the pathogen *Pyricularia grisea*. Isolates were obtained from lesions present on field-infected plants of different cultivars grown commercially in Uruguay and on experimental nurseries. Samples were collected during crop seasons from 1995 to 2001, both from infected leaves and neck-infected panicles. AFLPs markers were used to assess genomic diversity among *P. grisea* isolates. Isolates collected from different cultivars and different rice fields will be compared with isolates used for screening and selection of breeding lines with improved resistance under artificial inoculation. Using different strategies for characterization of representative samples of fungal isolates, combining anonymous marker loci (such as AFLPs) and DNA variation within fungal genomic sequences, is currently being applied to assess population structure of fungal isolates from Uruguayan rice fields.

Keywords: molecular, characterization, fingerprinting, AFLPs, ITS, *Pyricularia*, *Rhizoctonia*

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EVOLUCIÓN Y PREDICCIÓN DE GRADO DE SEVERIDAD DE PODREDUMBRE DEL TALLO Y MANCHA AGREGADA DE LAS VAINAS, MEDIANTE LA DETECCIÓN TEMPRANA DE SÍNTOMAS, EN TRES CULTIVARES

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Resultados anteriores de la investigación mostraron que es posible lograr buenos controles de Podredumbre del tallo y Mancha agregada de las vainas, provocadas por los hongos patógenos *Sclerotium oryzae* y *Rhizoctonia oryzae sativae*, mediante aplicaciones de fungicidas al inicio de floración. Se planteó el estudio de la evolución de las enfermedades del tallo en los cultivares más sembrados en Uruguay, el daño causado por las mismas en rendimiento en grano y calidad y la predicción de niveles de infección al final del ciclo basada en la detección temprana de síntomas, para establecer un umbral que justifique el control químico. Durante tres zafra (1994-1996), fueron instalados en Paso de la Laguna, Treinta y Tres, 15 ensayos con los cultivares Bluebelle, INIA Tacuarí y El Paso 144. En los mismos se realizó inoculación artificial de *Sclerotium oryzae* y *Rhizoctonia oryzae sativae* en dosis crecientes, incluyéndose un testigo con infección natural protegido con fungicida aplicado en floración y un testigo con infección natural sin fungicida. Los resultados de Mancha agregada de las vainas muestran que la severidad alcanzada por las parcelas inoculadas superan al testigo protegido en 46,6% para INIA Tacuarí, 27,2% para Bluebelle y 20,9% para El Paso 144. Eso implicó reducción del rendimiento en grano, de 12,0%, para INIA Tacuarí, 6,6% para Bluebelle y no significativa para El Paso 144. La misma comparación referida a la severidad de Podredumbre del tallo mostró valores de 23,5%, 37,0% y 23,6% respectivamente. Las correspondientes pérdidas de rendimiento en las parcelas inoculadas fueron 5,3% para INIA Tacuarí, 19,4% para Bluebelle y 5,7% para El Paso 144. Para ambas enfermedades, no hubieron diferencias en grado de severidad ni rendimiento entre los distintos niveles de inoculación. En los tres cultivares la evolución del índice de grado de severidad (Ou, 1986), creció a partir de la mitad de floración, con una evolución más rápida a partir de la etapa de doblado de la panoja en las dos enfermedades. No se logró establecer un umbral de infección, ya que en principio de floración los porcentajes de severidad alcanzados fueron muy bajos (0,5%).

Palabras clave: *Rhizoctonia oryzae sativae*, *Sclerotium oryzae*, control, inoculación, fungicida.

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STEM ROT AND AGGREGATE SHEATH SPOT EVOLUTION IN RICE AND DEGREE OF SEVERITY PREDICTION, THROUGH EARLY SYMPTOM DETECTION IN THREE CULTIVARS

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According with previous research results, it is possible to get good chemical controls of Stem rot and Aggregate sheath spot of rice, with fungicide applications in early flowering stage. The study of the development of that stem diseases in the three more used cultivars in Uruguay and yield and quality losses evaluation, was planned. The main objective was the prediction of final levels of infection based in the early symptom detection in order to establish a threshold to apply chemical control. During three growing seasons (1994-1996), fifteen experiments with the cultivars Bluebelle, INIA Tacuarí and El Paso 144 were installed in the experimental field of Paso de la Laguna, Treinta y Tres. Artificial inoculation with *Sclerotium oryzae* and *Rhizoctonia oryzae sativae* in increasing rates was made. A protected check with fungicide application in flowering stage and a check with natural infection without fungicide, was included. The results of Aggregate sheath spot showed that the disease severity reached at harvest time is higher in the inoculated plots than in the protected check in 46,6% for INIA Tacuarí, 27,2% for Bluebelle and 20,9% for El Paso 144. Those results generated a yield decrease of 12,0%, in INIA Tacuarí, 6,6% in Bluebelle and no differences for El Paso 144. The same comparison referred to the severity of Stem rot showed increments of 23.5%, 37.0% y 23.6% respectively. The corresponding yield losses were 5,3% for INIA Tacuarí, 19,4% for Bluebelle and 5,7% for El Paso 144. For both diseases, there were no differences among different levels of inoculation referred to disease severity and yield. The evolution of the Degree of severity index (Ou, 1986), of the studied diseases, developed after the middle of the flowering stage, with a higher increment at the end of that period and in the filling grain stage, for the three cultivars. The establishment of a threshold of infection was not possible because at the early flowering stage in Uruguayan climate conditions the disease severity levels were very low (0.5% or less).

Key-words: *Rhizoctonia oryzae sativae*, *Sclerotium oryzae*, chemical control, inoculation, fungicide.

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STUDIES OF *Sclerotium oryzae* AND *Rhizoctonia oryzae sativae* POPULATIONS IN THE SOIL, AND ITS RELATIONSHIP WITH THE RICE STEM DISEASES, IN URUGUAY

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Stem rot and Aggregate sheath spot caused by the pathogen fungi *Sclerotium oryzae* and *Rhizoctonia oryzae sativae*, developed into a major rice diseases in Uruguay during the last two decades. The study was conducted at INIA Treinta y Tres (33° Lat. S, 54° Long. W), from 1999 to 2002, to know the behavior of both fungi populations in the soil under different soil management and cultivars and their relationship with the diseases in the next crop. The pathogen density was determined through the soil sampling, in twelve selected sites. Each sample consisted of twenty cores of 3.0 cm in diameter and 12.0 cm in depth. In the sites, a stem disease severity index (Ou, 1986) present in the following crop was estimated. The sites selected were different in: crop frequencies (high and low), soil management (no tillage or conventional tillage) and cultivar planted (El Paso 144 and INIA Tacuarí). Soil inoculum density, (Number of sclerotium per g. of soil), increased in the sites with high crop frequency during the three growing seasons for *Sclerotium oryzae* ($p < 0.0001$) and only in the last period for *Rhizoctonia oryzae sativae* ($p = 0.0001$). The *Rhizoctonia* population density was higher in the sites with conventional tillage than in the sites with no tillage, during the growing season 2000-2001 ($p = 0.0001$) and more studies are necessary to confirm that result. The inoculum density was not affected by the cultivar planted, and changes were not detected between years for both pathogens. The pathogen density and the calculated disease severity Index of the next crop were positively associated for both fungi. In some situations, that correlation was not consistent through the years among different sites, due to different management practices.

Key-words: *Sclerotium oryzae*, *Rhizoctonia oryzae sativae*, population, density, soil fungi